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<p>(54) Title: PHARMACEUTICAL COMPOSITION, CONTAINING FRAGMENTS OF AN ANTIGENIC PROTEIN ENCODING DNA ENDOWED WITH ANTI-TUMOR EFFECT</p>		
<p>(57) Abstract</p> <p>Provided herein is a pharmaceutical composition containing one or more DNA molecules encoding fragments of a protein overexpressed in tumor cells, in order to induce an anti-tumor Ag-specific immune response, in association with suitable excipients and adjuvants.</p>		

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PHARMACEUTICAL COMPOSITION, CONTAINING FRAGMENTS OF AN ANTIGENIC PROTEIN ENCODING DNA ENDOWED WITH ANTI-TUMOR EFFECT.

#### Field of the invention

5       The invention relates to a pool of DNA plasmid constructs containing the sequences of human MUC-1 encoding fragments and to a pool of DNA plasmids in which the fragments themselves are preceded by the sequence encoding a protein consisting of human ubiquitin fused to a bacterial LacI fragment. The invention  
10       further relates to their use in the preparation of pharmaceutical compositions for use as DNA anti-tumor vaccines.

#### Background art

      The invention provides an anti-tumor therapy based on the induction or activation of the immune response able to bring  
15       about tumor rejection. The validity of such an idea is demonstrated from the first clinical results; for example, patients treated with a viral vaccine containing the Carcinoembryonic Antigen (CEA) encoding sequences demonstrated immune system activation against this antigen (Tsang KY et al.  
20       J. Natl. Cancer. Inst. 87: 982, 1995).

      The activation of an immune anti-tumor response is achievable through four different approaches:

- a) Ex vivo engineering of patient tumor cells in order to make them more immunogenic and suitable as a vaccine;
- 25       b) Ex vivo engineering of patient immune cells in order to pre-activate an *in vitro* immune response.
- c) Inoculation of naked or liposome capsulated or viral particle integrated (retrovirus, vaccinia virus, adenovirus, etc.) DNA encoding tumor associated antigens;
- 30       d) Treatment with recombinant or synthetic soluble tumor antigens conjugated or mixed with adjuvants.

      The first two approaches consist of the engineering of every single patient cell and are limited in that they are necessarily patient-specific, while the latter two are aimed to

obtain products comparable to a traditional drug.

The new vaccination methods reflect the development of new technologies. The recent indications coming from the experimentation on DNA naked vaccines that induce either a persistent antibody or a cell immune response, make the traditional protein subunit vaccines constituted of certain specific peptides, inducing a lymphocyte population, obsolete. Intramuscularly or intradermically injected proteins, encoded by naked DNA, induce a cytotoxic-specific response as well as a helper response. This powerful combination is extremely effective but the underling mechanism is not completely clarified yet. Muscle cells express class I MHC antigens at low levels only, and do not apparently express class II antigens or co-stimulatory molecules. Consequently, transfected muscle cells are unlikely to play an important role in the onset of the immune response per se. Recent data show that Antigen Presenting Cells (APC), such as macrophages or dendritic cells, play a fundamental role in capturing the myocyte released antigen and in the subsequent processing and presenting of the respective peptides in the context of the class I and II molecules, thus inducing a CD8+ cell activation with cytotoxic activity as well as activation of the CD4+ cells co-operating with B lymphocytes in eliciting the antibody response (Corr M et al *J. Exp. Med.* 184:1555, 1996) (Tighe, H. et al. *Immunology Today* 19:89, 1998).

Furthermore, the use of cytokines is known to improve the therapeutic effect deriving from immunization with DNA. Cytokines can be administered in the form of exogenous proteins as reported in Irvine et al., *J. Immunol.* 156: 238, 1996. An alternative approach is represented by the contemporaneous inoculation of both the tumor antigen or the desired cytokine encoding plasmids, thus allowing the cytokine to be produced *in situ* (Kim JJ et al. *Immunol* 158: 816, 1997).

The active immunization approach of the present invention is based on the use of DNA vectors as vaccines against the MUC-1

human antigen or Polymorphic Epithelial Mucin (PEM), overexpressed in tumor cells. MUC-1 is an epithelial luminal surface glycoprotein (Patton S. et al. *BBA* 1241:407, 1995). In the cell transformation process this glycoprotein loses the apical localization and its expression level rises dramatically. The protein function consists of protecting the luminal surfaces, for example in the mammal gland, ovary, endometrium, colon, stomach, pancreas, bladder, kidney, etc. A glycosylation defect is reported that makes tumor cell associated MUC-1 antigenically different from normal cell associated MUC-1. This phenomenon causes tumor MUC-1 to expose the antigen epitopes that are normally masked by the sugar moieties in the normal cell expressed MUC-1. This characteristic makes tumor MUC-1 particularly interesting in an induction of a tumor specific antibody response (Apostolopoulos V. et al. *Crit. Rev. Immunol.* 14:293, 1994).

As an objective, the vaccination is aimed at inducing immune responses against tumor cells expressing MUC1 at high levels, preserving at the same time the low expressing normal epithelia. The DNA vaccination relies upon the entrance of a gene or portions thereof inside the body cells followed by transcription and translation of the inserted sequence and thus the intracellular synthesis of the corresponding polypeptide. An important advantage of this system is that the neo-synthesized protein is naturally processed inside the cell and the produced peptides are associated with the Major Histocompatibility Complex class I molecules (MHC-I). The MHC/peptide complexes are therefore naturally exported to the cell surface where they can be recognized by the immune system CD8+ cytotoxic cells. Only the polypeptides synthesized inside the cell are then processed and presented in association with the MHC class I molecules, thus making it the only mechanism to stimulate, a specific cytotoxic response. Vaccination systems based on protein or peptide administration are usually more effective in stimulating

the antibody immune response which, to date, has been shown to be ineffective in rejecting tumor cells. Current gene therapy techniques rely upon DNA packaging in recombinant viral vectors (retrovirus and adenovirus). The naked DNA administration is much more advantageous in terms of effectiveness and safety compared to viral vector therapies (Kumar V and Sercarz E. *Nature Med.* 2: 857, 1996; McDonnell WM et al., *New England J. of Med.* 334: 42, 1996). In fact naked DNA is unable either to duplicate or integrate in the host tissue DNA and does not induce the immune response to viral proteins.

The use of the ubiquitin to enhance the neo-synthesized protein processing and thus cytotoxic lymphocyte induction was recently reported (Rodriguez F. et al., *J. Virology* 71: 8497, 1997). The use of ubiquitin in order to generate proteins with an N-terminal amino acid, making them unstable and thus prone to enhanced degradation, had been previously reported (Bechmair A. et al., *SCIENCE* 234: 179, 1986). The higher instability of these proteins was subsequently related to enhanced intracellular processing and presentation of model proteins by MHC-1 (Grant E P et al., *J. Immunol.* 155: 3750, 1995) (Wu Y and Kipps T.J., *J. Immunol.* 159: 6037, 1997).

The use of single constructs containing partial antigen encoding DNA fragments (influenza virus nucleoprotein), having a higher antigenic presentation efficiency compared to the analogues with the whole antigenic sequence, in DNA vaccination was reported (Anton L. C. et al., *J. Immunol.* 158: 2535, 1997). Furthermore the processing of intracellular proteins and presentation of the respective peptides by MHC class I proteins in physiologic conditions, underlie the mechanism of immunological surveillance. For a given protein and a specific MHC context, there are peptide fragments termed dominants (i. e. prevailing on subdominants or cryptics), which are unable to generate any immune response because they are recognized as "self". It has now been outlined, according to an aspect of the

present invention, that an approach aimed at supporting the non-dominant epitope presentation by the administration of a mix of antigen protein fragments is able to elicit a surprising cytotoxic immune response.

5        Description of the invention

It has now been found that DNA molecules, encoding fragments of a protein overexpressed in tumor cells, can be conveniently used to induce an antigen-specific anti-tumor immune response.

10        The invention relates particularly to a pharmaceutical composition containing one or more DNA encoding Mucin (MUC-1) protein fragments.

The DNA used in the present invention can be plasmid or viral DNA, preferably plasmid DNA obtained employing the pMRS30 expression vector described in fig. 13.

15        The compositions according to the invention contain preferably at least two DNA fragments of the Mucin (MUC-1) or of another protein overexpressed in tumor cells.

The compositions according to the invention contain preferably at least four fragments, each ranging from 200 to about 700 nucleotides, each sequence being juxtaposed and possibly partially overlapping, from about 50 to about 150 nucleotides, at the 3' and/or 5' end of the adjacent one.

20        The DNA fragments according to the invention can be possibly preceded at the 5' end by a ubiquitin encoding DNA sequence and possibly also by a LacI portion of Escherichia coli.

The invention relates also to new DNA fragments and to the use of Mucin-1 fragments defined above in the medicine and anti-tumor vaccine preparation.

30        Description of the figures

Fig. 1

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS166 expression

vector. This DNA includes the sequence corresponding to nucleotides 136-339 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by the two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 136-339 fragment of the EMBL sequence J05581.

**Fig. 2**

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS169 expression vector. This DNA includes the sequence corresponding to nucleotides 205-720 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 205-720 fragment of the EMBL sequence J05581.

**Fig. 3**

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS168 expression vector. This DNA includes the sequence corresponding to nucleotides 631-1275 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 631-1275 fragment of the EMBL sequence J05581.

**Fig. 4**

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS167 expression vector. This DNA includes the sequence corresponding to nucleotides 1222-1497 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the

amino acids encoded by the 1222-1497 fragment of the EMBL sequence J05581.

Fig. 5

5 Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS175 expression vector. This DNA includes the sequence corresponding to nucleotides 136-1497 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The  
10 encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 136-1497 fragment of the EMBL sequence J05581.

Fig. 6

15 Nucleotide DNA sequence (with the respective amino acid sequence) termed UBILacI. The encoded polypeptide includes the Ubiquitin sequence fused to a partial sequence of the bacterial protein beta-galactosidase, as described in *Chau V. et al. Science 243: 1576, 1989.*

Fig. 7

20 Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the expression vector pMRS30 to give the pMRS171 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence corresponding to nucleotides 136-339 of the EMBL sequence J05581  
25 followed by two translation stop codons, TGA and TAA. The coded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 136-339 of the EMBL sequence J05581.

Fig. 8

30 Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS174 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 205-720 of the EMBL

sequence J05581 followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 205-720 of the EMBL sequence J05581.

#### Fig. 9

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS173 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 631-1275 of the EMBL sequence J05581 followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 631-1275 of the EMBL sequence J05581.

#### Fig. 10

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS172 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 1222-1497 of the EMBL sequence J05581 followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 1222-1497 of the EMBL sequence J05581.

#### Fig. 11

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS176 expression vector. This DNA includes the sequence named UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 136-1497 of the EMBL sequence J05581 followed by two translation stop codons, TGA and

TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 136-1497 of the EMBL sequence J05581.

Fig. 12

Electrophoretic analysis on 1% agarose gel in 1X TBE. mRNA extracted from CHO, CD34+ dendritic cells and dendritic cells from PEMC, respectively, transfected with pMRS169, and subjected to RT-PCR reaction either with (lanes 4, 8, 12) or without (lanes 5, 9, 13) Reverse Transcriptase. Molecular weight DNA marker (lane 1); internal negative controls (lanes 2, 6); internal positive controls (lanes 3, 7, 10, 11); positive control from Promega kit (lane 14).

Fig. 13

Nucleotide sequence of the pMRS30 expression vector. The 1-2862 region corresponds to the AccI (location 504) - BamHI (location 3369) region of the pSV2CAT vector (EMBL M77788); the 2863-3721 region includes the human cytomegalovirus promoter (human cytomegalovirus major immediate-early gene enhancer); the 3722-4905 region includes several cloning sites, including XbaI (location 3727), and the processing signal of the rabbit beta-globin gene.

Detailed description of the invention

A DNA plasmid pool encoding, in eukaryotic cells, fragments of the MUC-1 human protein antigen was prepared. Constructs are based on the mammalian expression vector termed pMRS30, described in figure 13 and previously claimed in the Patent Application WO95/11982, and contain partial sequences of the MUC-1 cDNAs reported in the EMBL database with accession number J05581. MUC-1 encoding DNA was fragmented so that each fragment represents a discrete portion, partially overlapping to the adjacent ones. Administration of a mix of such plasmids can cause different plasmids to transfect different APC cells at the administration site. Therefore such cells produce and process

discrete portions of the MUC-1 protein giving the related peptides. In those conditions, the occurring subdominant and cryptic peptides can also be presented in association with class I MHC molecules thus generating a cytotoxic immune response.

5       The present invention thus relates to the use of a group of four constructs (Figures 1 to 4) containing MUC-1 cDNA partial fragments in admixture containing at least two of them and a group of four constructs (Figures 7 to 10) containing MUC-1 cDNA partial fragment preceded by the DNA encoding a protein sequence  
10       containing Ubiquitin and an Escherichia coli Lac I portion (Figure 6) used separately or in admixture containing at least two of them.

      The present invention relates also to the use of the construct (Figure 5) containing the almost complete sequence of  
15       the MUC-1 cDNA and the construct (Figure 11) containing the almost complete sequence of the MUC-1 cDNA preceded by the DNA encoding a protein sequence containing Ubiquitin and an Escherichia coli Lac I portion.

      The mixture of the four constructs containing the partial  
20       fragments of the MUC-1 cDNA and the mixture of the four constructs containing the partial fragments of the MUC-1 cDNA preceded by the DNA encoding a protein sequence, containing Ubiquitin and an Escherichia coli Lac I portion, represents a preferred embodiment of the present invention.

25       Constructs according to the present invention can be used in the anti-tumor therapy of patient affected with tumors characterized by high MUC-1 expression.

      Constructs described in the present invention were obtained as follows.

30       In the case of the first series of constructs, the fragments of the MUC-1 DNA were obtained by RT-PCR from BT20 cell line or by DNA partial chemical synthesis. Such fragments were then cloned into the pMRS30 expression vector and verified by sequencing.

In the case of the second series of constructs, the fragments were obtained from the first series of constructs by a PCR re-amplification. These fragments were then fused to the DNA encoding the Ubiquitin (obtained by RT-PCR from MCF7 cell line mRNA) and a partial lacI sequence (obtained by PCR from the commercial vector pGEX). DNA sequences thus obtained were then cloned in the pMRS30 expression vector and verified by sequencing. For the intended therapeutic or prophylactic uses, fragments or constructs according to the invention are suitably formulated, using carriers and methods previously employed in naked DNA vaccines, as described for example in The Immunologist, 1994, 2:1; WO 90/11092, Proc. Natl. Acad. Sci. U.S.A., 1986, 83, 9551; US 5580859; Immunology today 19 (1998), 89-97; Proc. Natl. Acad. Sci. U.S.A. 90 (1993), 11478-11482; Nat. Med. 3 (1997), 526-532; Vaccine 12 (1994), 1495-1498; DNA Cell. Biol. 12 (1993), 777-783. The dosages will be determined on the basis of clinical and pharmacological-toxicological trials. Generally speaking, they will be comprised between 0.005 µg/kg and 5 µg/kg of the fragment mix. The composition of the invention can also contain a cytokine or a cytokine encoding plasmid.

The invention will be further illustrated by means of the following examples.

**Example 1. Plasmid pMRS166 construction.**

BT20 tumor cells (ATCC HTB-19) were cultured in Eagles MEM supplemented with 10% fetal calf serum. Ten million cells were trypsinized, washed with PBS, and mRNA extracted.

An aliquot of this RNA was subjected to RT-PCR (reverse transcriptase-polymerase chain reaction) reaction in the presence of the following synthetic oligonucleotides:

V11 (5 GATCTCTAGAAATGACAGGTTCTGGTCATGCAAGC 3)

V4 (5 GATCTCTAGAAAGCTTATCAACCTGAAGCTGGTTCGGTGGC 3)

The produced DNA fragment, purified and digested with the restriction enzyme XbaI, was cloned into the pMRS30 expression

vector, containing the human cytomegalovirus promoter and the beta-globin polyadenylation signal as claimed in the Patent WO9511982. The resulting pMRS166 vector contains a DNA fragment including the ATG codon, the sequence corresponding to the nucleotides 136-339 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 1.

**Example 2. Plasmid pMRS169 construction.**

An aliquot of the RNA obtained as reported in example 1 was amplified by RT-PCR in the presence of the following synthetic oligonucleotides:

V12 (5 GATCTCTAGAATGGTGCCCGCTCTACTGAGAAGAATGC 3)

V15 (5 GGCGGTGGAGCCCGGGCTGGCTTGT 3)

The produced DNA fragment, purified and digested with the restriction enzymes SmaI and XbaI, was fused, by the SmaI restriction site, to a DNA fragment entirely synthetically constructed, and including a sequence partially corresponding to the nucleotides 457-720 of the EMBL sequence J05581 and two stop codons, TGA and TAA. The whole fragment was thus cloned in the XbaI site of the pMRS30 expression vector. The resulting pMRS169 vector contains a DNA fragment including the ATG codon, the sequence partially corresponding to the nucleotides 205-720 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 2.

**Example 3. Plasmid pMRS168 construction.**

An aliquot of the RNA obtained as reported in example 1 was amplified by RT-PCR in the presence of the following synthetic oligonucleotides:

V13 (5 GATCTCTAGAATGGGCTCAGCTTCTACTCTGGTGACACACGGC 3)

V8 (5 GATCTCTAGAAAGCTTATCACAAAGCAATGAGATAGACAATGGCC 3)

The produced DNA fragment, purified and digested with the restriction enzyme XbaI was cloned in the pMRS30 expression vector. The resulting pMRS168 vector contains a DNA fragment including the ATG codon, the sequence corresponding to the

nucleotides 631-1275 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 3.

**Example 4. Plasmid pMRS167 construction.**

5 An aliquot of the RNA obtained as reported in example 1 was subjected to RT-PCR reaction in the presence of the following synthetic oligonucleotides:

V14 (5 GATCTCTAGAATGCTGGTCTGGTCTGTGTCTGGTTGCGC 3)

V10 (5 GATCTCTAGAAAGCTTATCACAAGTTGGCAGAAGTGGCTGC 3)

10 The produced DNA fragment, purified and digested with the restriction enzyme XbaI was cloned in the pMRS30 expression vector. The resulting pMRS167 vector contains a DNA fragment including the ATG codon, the sequence corresponding to the nucleotides 1222-1497 of the EMBL sequence J05581, and two stop  
15 codons, TGA and TAA.

This fragment is reported in fig. 4.

**Example 5. Plasmid pMRS175 construction.**

pMRS166, 169, 168, 167 plasmids were subjected to PCR reaction in the presence of the following nucleotide pairs:

20 V11 (see example 1)

V18 (5 AACCTGAAGCTGGTCCGTGGC 3) for pMRS166

V19 (5 GTGCCCAGCTCTACTGAGAAGAATGC 3)

V20 (5 GCTGGGAATTGAGAATGGAGTGCTCTTGC 3) for pMRS169

V21 (5 GGCTCAGCTTCTACTCTGGTGACACACGCG 3)

25 V22 (5 CAAGGCAATGAGATAGACAATGGCC 3) for pMRS168

V23 (5 CTGGTGCTGGTCTGTGTTCTGGTTGCG 3)

V10 (see example 4) for pMRS167

The four DNA fragments obtained in the respective PCR reactions were mixed in equimolar amounts and PCR reacted in the  
30 presence of the V11 and V10 oligonucleotides.

The produced DNA fragment, purified and digested with the XbaI restriction enzyme, was cloned in the pMRS30 expression vector. The resulting pMRS175 vector contains a DNA fragment including the ATG codon, the sequence partially corresponding to

the nucleotides 136-1497 of the EMBL sequence J05581 and two stop codons TGA and TAA.

This fragment is reported in fig. 5.

**Example 6. Plasmid pMRS171 construction.**

5 MCF7 tumor cells (ATCC HTB-22) were cultured in Eagles MEM supplemented with 10% fetal calf serum. Ten million cells were trypsinized, washed with PBS, and mRNA extracted.

An aliquot of this RNA was subjected to RT-PCR in the presence of the following synthetic oligonucleotides:

10 UBIup (5GATCTCTAGAATGCAGATCTTCGTGAAGACCCCTGACTGGT 3)

UBIdown

(5TCACCAGCGAGACGGGCAACAGCCATGCACCACTACCGTGCTCCCACTCTGAGACGGAGC  
ACCAGG 3)

The reaction produces a DNA fragment termed fragment 1.

15 DNA from pGEX11T (Pharmacia) was subjected to PCR reaction in the presence of the following synthetic oligonucleotides:

LacIup (5CCTCCGTCTCAGAGGTGGGAGGACGGTAGTGGTGATGGCTGTTGCC  
GTCTCGCTGGTGAAAAG 3)

LacIdown (5GATCGGATCCTCGGGAAACCTGTCTGCCAGCTGC 3)

20 This reaction gives a DNA fragment termed fragment 2.

The 1 and 2 DNA fragments, obtained in the respective PCR reactions, were mixed in equimolar amounts and subjected to PCR reaction in presence of the UBIup and LacIdown oligonucleotides.

25 The produced DNA fragment, purified and digested with the restriction enzymes XbaI and BamHI, was cloned into the pUC18 commercial plasmid. The resulting pMRS156 vector contains a DNA fragment including the sequence encoding the ubiquitin fused to the sequence encoding a bacterial beta-galactosidase portion. This fragment, termed UBILacI, is reported in fig. 6.

30 Plasmid pMRS166 DNA was subjected to a PCR reaction in presence of the following synthetic oligonucleotides:

V3 (5GATCGGATCCACAGGTTCTGGTCATGCAAGC 3)

V4 (see Example 1)

The produced DNA fragment, purified and digested with the

restriction enzymes XbaI and BamHI, was fused, by ligation into the two BamHI sites, to the UBILacI fragment deriving from the pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS171 vector contains  
5 a DNA fragment including the UBILacI sequence, the sequence corresponding to the 136-339 nucleotides of the EMBL sequence J05581 and two stop codons, TGA and TAA. This fragment is reported in fig. 7.

**Example 7. Plasmid pMRS174 construction.**

10 Plasmid pMRS169 DNA was subjected to PCR reaction in the presence of the following synthetic oligonucleotides:

V5 (5GATCGGATCCGTGCCAGCTCTACTGAGAAGATGC 3)

V6 (5GATCTCTAGAAAGCTTATCAGCTGGGAATTGAGAATGGAGTGCTCTTGC 3)

The produced DNA fragment, purified and digested with the  
15 restriction enzymes XbaI and BamHI, was fused, by ligation into the two BamHI sites, to the UBILacI fragment deriving from the pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS174 vector contains a DNA fragment including the UBILacI sequence, the sequence  
20 corresponding to the 205-720 nucleotides of the EMBL sequence J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 8.

**Example 8. Plasmid pMRS173 construction.**

Plasmid pMRS168 DNA was subjected to PCR reaction in the  
25 presence of the following synthetic oligonucleotides:

V7 (5GATCGGATCCGGCTCAGCTTCTACTCTGGTGACACACGGC 3)

V8 (see example 3)

The produced DNA fragment, purified and digested with the  
restriction enzymes XbaI and BamHI, was fused, by ligation into  
30 the two BamHI sites, to the UBILacI fragment deriving from the pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS173 vector contains a DNA fragment including the UBILacI sequence, the sequence corresponding to the 631-1275 nucleotides of the EMBL sequence

J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 9.

**Example 9. Plasmid pMRS172 construction.**

Plasmid pMRS167 DNA was subjected to PCR reaction in the presence of the following synthetic oligonucleotides:

V9 (5 GATCGGATCCCTGGTGTCTGGTCTGTGTTGTTGCGC 3)

V10 (see example 4)

The produced DNA fragment, purified and digested with the restriction enzymes XbaI and BamHI, was fused, by ligation into the two BamHI sites, to the UBILacI fragment deriving from pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS172 vector contains a DNA fragment including the UBILacI sequence, the sequence corresponding to the 1222-1497 nucleotides of the EMBL sequence J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 10.

**Example 10. Plasmid pMRS176 construction.**

Plasmid pMRS167 DNA was subjected PCR reaction in the presence of the following synthetic oligonucleotides:

V3 (see example 6)

V10 (see example 4)

The produced DNA fragment, purified and digested with the restriction enzymes XbaI and BamHI, was fused, by ligation into the two BamHI sites, to the UBILacI fragment deriving from pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS176 vector contains a DNA fragment including the UBILacI sequence, the sequence corresponding to the 136-1497 nucleotides of the EMBL sequence J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 11.

**Example 11. Eukaryotic cell transfection and testing for transcription.**

CHO (Chinese Hamster Ovary) cells were cultured in alpha MEM supplemented with ribonucleotides and deoxyribonucleotides

at transfection time.

Dendritic cells were obtained from CD34+ hemopoietic precursors cultured in IMDM without serum, supplemented with GM-CSF, IL4, SCF, Flt3 and TNFalpha. After 7 days the obtained cell  
5 population was transfected.

Dendritic cells were obtained from monocytes isolated from PBMC (peripheral blood mononuclear cells), cultured in RPMI supplemented with FCS, GM-CSF, and IL-4. After 7 days the obtained cell population was transfected.

10 In each case, about one million cells were transfected with one of the plasmids reported in examples 1 to 10. Transfection was carried out using 3 µg of plasmid DNA and 4 µl of DMRIE (Gibco) by lipofection.

After 24 hours cells were harvested, washed with PBS and  
15 lysed in order to extract the mRNA.

A mRNA aliquot was subjected to RT-PCR reaction in the presence of the oligonucleotide pair specific for the transfected DNA plasmid.

This experiment was carried out for each plasmid reported  
20 in the examples 1 to 10, using the following oligonucleotide pairs: V11/V4 for pMRS166, V12/V6 for pMRS169, V13/V8 for pMRS168, V4/V10 for pMRS167, V4/V10 for pMRS175, UBIup/V4 for pMRS171, UBIup/V6 for pMRS174, UBIup/V8 for pMRS173, UBIup/V10 for pMRS172, V14/V10 for pMRS176.

25 As a representative example, figure 12 reports the electrophoretic analysis of the DNA fragments obtained by RT-PCR from the mRNA of the three cell populations, transfected with the pMRS169 plasmid. In this case the oligonucleotide pair V12/V6 was used.

30 **Example 12. In vivo study results.**

In the in vivo studies, the mixtures of the four fragments and the pMRS30 plasmid (vector without insert and thus used as a negative control) were used. In order to test the occurred immunization, an ELISA test was used to show the human mucin

specific antigens.

The *in vivo* studies were conducted using human MUC1 transgenic C57BL mice. As a consequence in these animals the MUC1 protein represents a self-protein. The employed vaccination  
5 schedule consists of 3 intradermic (dorsal portion, 50 micrograms DNA for each side) administrations (at days 0, 14, 28) of 100 micrograms plasmid DNA. At day 14 after the last administration, the animals were sacrificed and sera were tested for anti-human mucin antibodies.

10 The assayed fragment mixes, object of the present invention, stimulated a good immune response in the treated animals.

On the other hand, vaccination experiments with a 60-aminoacid peptide corresponding to the 20 aminoacids reported in  
15 fig. 2, from location 86 to location 105, repeated three times (this peptide is termed 3XTR), were also carried out.

The two vaccinations differ in the type of the elicited antibody response. The antibody titer results much more higher in the vaccination with 3XTR. Furthermore the noticed IgG  
20 subtypes are in favor of an essentially humoral (antibody) response in the case of vaccination with 3XTR, and of a cellular response (cytotoxic) in the case of vaccination with DNA. For anti-tumor therapy, a principally cytotoxic immune response is preferable. Because the experiments were carried out on  
25 transgenic mice, in whom the human mucin is "self", we can foresee a similar response in humans. This response could justify the use, as DNA vaccines, of the compounds of the present invention in the treatment of MUC1 overexpressing human tumors.

CLAIMS

1. Pharmaceutical composition containing one or more DNA molecules, encoding fragments of a protein overexpressed in tumor cells in order to induce an antitumor Ag-specific immune response, in combination with suitable excipients and adjuvants.
2. Pharmaceutical composition according to claim 1 wherein the overexpressed protein is MUC-1.
3. Pharmaceutical composition according to claim 1 or 2 containing at least two DNA molecules each containing a cDNA sequence encoding a Mucin fragment (MUC-1).
4. Composition according to claim 3 containing at least three DNA molecules each containing a cDNA sequence encoding a Mucin fragment (MUC-1).
5. Composition according to claim 4 containing at least four DNA molecules each containing a cDNA sequence encoding a Mucin fragment (MUC-1).
6. Composition according to claims 3, 4 or 5 wherein the DNA sequences comprise about 200 to about 700 nucleotides, each sequence being contiguous and possibly partially overlapping, from about 50 to about 150 nucleotides at the 3' and/or 5' end, to the adjacent one.
7. Pharmaceutical composition according to any claim from 2 to 6 wherein the used mixture consists of, at least, two plasmid DNA molecules, each containing a DNA fragment selected from those whose sequences are described in figures 1, 2, 3, and 4.
8. Pharmaceutical composition according to claim 7 wherein the used mixture consists of the pool of plasmid DNA molecules, where each molecule contains a DNA fragment selected from those whose sequences are described in figures 1, 2, 3, and 4.
9. Pharmaceutical composition according to claim 1 or 2 wherein a plasmid DNA molecule containing the sequence described in figure 5 is used.
10. Pharmaceutical composition according to claims 7, 8, or 9

wherein the used plasmid DNA molecules derive from the fusion of the pMRS30 expression vector in Fig. 13 to each sequence described in figures 1, 2, 3, 4, 5.

11. Pharmaceutical composition according to claims 2 to 6  
5 wherein the used sequences, corresponding to single fragments of the protein, are preceded in the 5' termini by the sequence described in Fig. 6 encoding the ubiquitin and a LacI portion from Escherichia Coli.
12. Pharmaceutical composition according to claim 11 wherein the  
10 mixture consists of one or more sequences deriving from joining the pMRS30 expression vector, described in Fig. 13, to a DNA sequence selected from those described in figures 7, 8, 9, and 10.
13. Pharmaceutical composition according to claim 11 wherein the  
15 mixture consists of the totality of the sequences deriving from joining the pMRS30 expression vector to a DNA sequence selected from those described in figures 7, 8, 9, and 10.
14. Pharmaceutical composition according to claim 11 wherein the  
mixture consists of a sequence deriving from joining the pMRS30  
20 expression vector to the sequence described in figure 11.
15. Pharmaceutical composition according to any preceding claims, further containing a cytokine or a cytokine encoding plasmid.
16. A plasmid DNA molecule consisting of the pMRS30 expression  
25 vector joined to a DNA sequence, encoding a MUC-1 protein fragment and whose sequence is selected from the group of those described in figures 1, 2, 3, 4, and 5.
17. A DNA molecule encoding a protein MUC-1 fragment preceded in its 5' terminus by the sequence described in Fig. 6.
18. A DNA molecule according to claim 17 selected from those  
30 described in figures 7, 8, 9, 10, and 11.
19. A plasmid DNA molecule obtained by joining the pMRS expression vector to a DNA molecule selected from those of claim 17 or 18.

20. Use of DNA molecules of claims 16-19 in the preparation of a composition with anti-tumor effect.

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Figure 1

```
1  ATGACAGGTTCTGGTCATGCAAGCTCTACCCCAGGTGGAGAAAAG
1▶ Met Thr Gl ySer Gl yHi sAl aSer Ser Thr ProGl yGl yGl uLys
46  GAGACTTCGGCTACCCAGAGAAGTTCAGTGCCCAGCTCTACTGAG
16▶ Gl uThr Ser Al aThr Gl nArgSer Ser Val ProSer Ser Thr Gl u
91  AAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGCCACAGC
31▶ LysAsnAl aVal Ser Met Thr Ser Ser Val LeuSer Ser Hi sSer
136 CCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTCACCTCTG
46▶ ProGl ySer Gl ySer Ser Thr Thr Gl nGl yGl nAspVal Thr Leu
181 GCCCCGGCCACGGAACCACTTCAGGTTGATAA
61▶ Al aProAl aThr Gl uProAl aSer Gl y.....
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Figure 2

1 ATGGTGCCAGCTCTACTGAGAAGAATGCTGTGAGTATGACCAGC  
1▶ Met Val Pro Ser Ser Thr Glu Lys Asn Ala Val Ser Met Thr Ser  
46 AGCGTACTCTCCAGCCACAGCCCCGGTTCAGGCTCCTCCACCACT  
16▶ Ser Val Leu Ser Ser His Ser Pro Gly Ser Gly Ser Ser Thr Thr  
91 CAGGGACAGGATGTCACTCTGGCCCCGGCCACGGAACAGCTTCA  
31▶ Gl n Gly Gl n Asp Val Thr Leu Ala Pro Ala Thr Glu Pro Ala Ser  
136 GGTTCAGCTGCCACCTGGGGACAGGATGTCACTCGGTCCCAGTC  
46▶ Gly Ser Ala Ala Thr Trp Gly Gl n Asp Val Thr Ser Val Pro Val  
181 ACCAGGCCAGCCCTGGGCTCCACCACCCGCGCCAGCCACGATGTC  
61▶ Thr Arg Pro Ala Leu Gly Ser Thr Thr Pro Pro Ala His Asp Val  
226 ACCTCAGCCCCGGACAACAAGCCAGCCCCGGGAAGTACTGCTCCA  
76▶ Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro Gly Ser Thr Ala Pro  
271 CCAGCACACGGTGTTACCTCGGCTCCGATACCAGGCCGGCCCCA  
91▶ Pro Ala His s Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro  
316 GGTAGTACCGCCCCCTCCTGCCCATGGTGTCACTCTGCCCCGGAC  
106▶ Gly Ser Thr Ala Pro Pro Ala His s Gly Val Thr Ser Ala Pro Asp  
361 AACAGGCCTGCATTGGGTAGTACAGCACCGCCAGTACACAACGTT  
121▶ Asn Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His Asn Val  
406 ACTAGTGCCTCAGGCTCTGCTAGCGGCTCAGCTTCTACTCTGGTG  
136▶ Thr Ser Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu Val  
451 CACAACGGCACCTCTGCGCGCGGACCACAACCCAGCGAGCAAG  
151▶ His Asn Gly Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys  
496 AGCACTCCATTCTCAATTCCAGCTGATAA  
166▶ Ser Thr Pro Phe Ser Ile Pro Ser .....

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Figure 3

1 ATGGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCTGCCAGG  
 1▶ Met Gly Ser Ala Ser Thr Leu Val His Asn Gly Thr Ser Ala Arg  
 46 GCTACCACAACCCAGCCAGCAAGAGCACTCCATTCTCAATTCCC  
 16▶ Ala Thr Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe Ser Ile Pro  
 91 AGCCACCCTCTGATACTCCTACCACCCTTGCCAGCCATAGCACC  
 31▶ Ser His His Ser Asp Thr Pro Thr Thr Leu Ala Ser His Ser Thr  
 136 AAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCTCCTCTC  
 46▶ Lys Thr Asp Ala Ser Ser Thr His His Ser Thr Val Pro Pro Leu  
 181 ACCTCCTCCAATCACAGCACTTCTCCCAGTTGTCTACTGGGGTC  
 61▶ Thr Ser Ser Asn His Ser Thr Ser Pro Gl n Leu Ser Thr Gl y Val  
 226 TCTTTCTTTTCTGTCTTTTCACATTCAACCTCCAGTTTAAT  
 76▶ Ser Phe Phe Phe Leu Ser Phe His Ile Ser Asn Leu Gl n Phe Asn  
 271 TCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAGCTGCAG  
 91▶ Ser Ser Leu Gl u Asp P ro Ser Thr Asp Tyr Tyr Gl n Gl u Leu Gl n  
 316 AGAGACATTTCTGAAATGTTTTTGCAGATTTATAAACAGGGGGT  
 106▶ Arg Asp Ile Ser Gl u Met Phe Leu Gl n Ile Tyr Lys Gl n Gl y Gl y  
 361 TTTCTGGGCTCTCCAATATTAAGTTCAGGCCAGGATCTGTGGTG  
 121▶ Phe Leu Gl y Leu Ser Asn Ile Lys Phe Arg Pro Gl y Ser Val Val  
 406 GTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAATGTGCCA  
 136▶ Val Gl n Leu Thr Leu Ala Phe Arg Gl u Gl y Thr Ile Asn Val His  
 451 GACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCAGCCTCT  
 151▶ Asp Val Gl u Thr Gl n Phe Asn Gl n Tyr Lys Thr Gl u Ala Ala Ser  
 496 CGATATAACCTGACGATCTCAGACGTGACGTGAGTGATGTGCCA  
 166▶ Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val Pro  
 541 TTTCTCTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGCTGGGGC  
 181▶ Phe Pro Phe Ser Ala Gl n Ser Gl y Ala Gl y Val Pro Gl y Trp Gl y  
 586 ATCGCGCTGCTGGTGTCTGGTCTGTGTCTGGTTGCGCTGGCCATT  
 196▶ Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala Leu Ala Ile  
 631 GTCTATCTCATTGCCTTGTGATAA  
 211▶ Val Tyr Leu Ile Ala Leu.....

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Figure 4

```
1  ATGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATTGTCTAT
1▶Met LeuVal LeuVal CysVal LeuValAlaLeuAlaIleValTyr
46 CTCATTGCCCTGGCTGTCTGTCA GTGCCGCCGAAAGAACTACGGG
16▶LeuIleAlaLeuAlaVal CysGlnCysArgArgLysAsnTyrGly
91 CAGCTGGACATCTTTCCAGCCCGGATACCTACCATCCTATGAGC
31▶GlnLeuAspIlePheProAlaArgAspThr TyrHisProMetSer
136 GAGTACCCACCTACCACACCCATGGGCGCTATGTGCCCCCTAGC
46▶GluTyrProThr TyrHisThrHisGlyArgTyrValProProSer
181 AGTACCGATCGTAGCCCTATGAGAAGGTTTCTGCAGGTAATGGT
61▶SerThrAspArgSerProTyrGluLysValSerAlaGlyAsnGly
226 GGCAGCAGCCTCTCTTACACAAACCCAGCAGTGGCAGCCACTTCT
76▶GlySerSerLeuSerTyrThrAsnProAlaValAlaAlaThrSer
271 GCCAACTTGTGATAA
91▶AlaAsnLeu*****
```

Figure 5

1 ATGACAGGTTCTGGTCATGCAAGCTCTACCCAGGTGGAGAAAAG  
 1▶ Met Thr Gl ySer Gl yHisAl aSer Ser Thr ProGlyGlyGlyLys  
 46 GAGACTTCGGCTACCCAGAGAAGTTCAAGTGCACGCTCTACTGAG  
 16▶ Gl uThr Ser Al aThr Gl nArgSer Ser Val ProSer Ser Thr Gl u  
 91 AAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGCCACAGC  
 31▶ LysAsnAl aVal Ser Met Thr Ser Ser Val LeuSer Ser HisSer  
 136 CCCGGTTCAGGCTCCTCCACCCTCAGGGACAGGATGTCACTCTG  
 46▶ ProGlySer Gl ySer Ser Thr Thr Gl nGlyGly nAspVal Thr Leu  
 181 GCCCGGCCACGGAACCAGCTTCAGGTTCAAGTTCAGCTGCCACCTGGGGA  
 61▶ Al aProAl aThr Gl uProAl aSer Gl ySer Al aAl aThr TrpGly  
 226 CAGGATGTCACTCGTCCAGTCAACAGGCCAGCCCTGGGCTCC  
 76▶ Gl nAspVal Thr Ser Val ProVal Thr ArgProAl aLeuGlySer  
 271 ACCACCCCGCCAGCCAGATGTCACTCAGCCCGGACAACAAG  
 91▶ Thr Thr ProProAl aHisAspVal Thr Ser Al aProAspAsnLys  
 316 CCAGCCCCGGGAAGTACCGTCCACCAGCACACGGTGTACCTCG  
 106▶ ProAl aProGlySer Thr Al aProProAl aHisGlyVal Thr Ser  
 361 GCTCCGGATACCAGGCCGGCCCGAGGTAGTACCGCCCTCTCTGCC  
 121▶ Al aProAspThr ArgProAl aProGlySer Thr Al aProProAl a  
 406 CATGTGTACATCTGCCCGGACAACAGGCCTGCATTGGGTAGT  
 136▶ HisGlyVal Thr Ser Al aProAspAsnArgProAl aLeuGlySer  
 451 ACAGCACCGCCAGTACACAACGTTACTAGTGCCTCAGGCTCTGCT  
 151▶ Thr Al aProProVal HisAsnVal Thr Ser Al aSer Gl ySer Al a  
 496 AGCGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCTGCGCGC  
 166▶ Ser Gl ySer Al aSer Thr LeuVal HisAsnGlyThr Ser Al aArg  
 541 GCGACCACAACCCAGCGAGCAAGAGCACTCCATTCTCAATTCCC  
 181▶ Al aThr Thr Thr ProAl aSer LysSer Thr ProPheSer IlePro  
 586 AGCCACCACTCTGATACTCTACCACCCTTGCCAGCCATAGCACC  
 196▶ Ser HisShi sSer AspThr ProThr Thr LeuAl aSer HisSer Thr  
 631 AAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCTCTCTC  
 211▶ LysThr AspAl aSer Ser Thr HisShi sSer Thr Val ProProLeu  
 676 ACCTCTCCAATCACAGCACTTCTCCCCAGTTGTCTACTGGGGTC  
 226▶ Thr Ser Ser AsnHisSer Thr Ser ProGly nLeuSer Thr Gl yVal  
 721 TCTTTCTTTTCTCTGTCTTTTTCACATTTCAAACCTCCAGTTTAAT  
 241▶ Ser PhePhePheLeuSer PheHisIleSer AsnLeuGly nPheAsn  
 766 TCCTCTCTGGAAGATCCAGCACCGACTACTACCAAGAGCTGCAG  
 256▶ Ser Ser LeuGly uAspProSer Thr AspTyrTyrGly nGly uLeuGly n  
 811 AGAGACATTTCTGAAATGTTTTTGACAGATTTATAACAAGGGGGT  
 271▶ ArgAspIleSer Gl uMetPheLeuGly nIleTyrLysGly nGly yGly  
 856 TTTCTGGGCCTCTCCAATATTAAGTTCAAGCCAGGATCTGTGGTG  
 286▶ PheLeuGlyLeuSerAsnIleLysPheArgProGlySer Val Val

(Continued)

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Figure 5 (continued)

901 GTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAATGTCCAC  
 301▶ Val Gl nLeuThr LeuAl aPheArgGl uGl yThr l l eAsnVal l Hi s  
 946 GACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCAGCCTCT  
 316▶ AspVal l Gl uThr Gl nPheAsnGl nTyrLysThr Gl uAl aAl aSer  
 991 CGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGATGTGCCA  
 331▶ ArgTyrAsnLeuThr l l eSerAspVal Ser Val SerAspVal Pro  
 1036 TTTCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGCTGGGGC  
 346▶ PheProPheSer Al aGl nSer Gl yAl aGl yVal l ProGl yTrpGl y  
 1081 ATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATT  
 361▶ l l eAl aLeuLeuVal l LeuVal CysVal l LeuVal Al aLeuAl a l l e  
 1126 GTCTATCTCATTGCCTTGGCTGTCTGTCTCAGTGCCGCCGAAAGAAC  
 376▶ Val TyrLeu l l eAl aLeuAl aVal CysGl nCysArg ArgLysAsn  
 1171 TACGGGCAGCTGGACATCTTTCCAGCCCGGGATACCTACCATCCT  
 391▶ TyrGl yGl nLeuAsp l l ePheProAl aArgAspThr TyrHi sPro  
 1216 ATGAGCGAGTACCCACCTACCACACCCATGGGCGCTATGTGCCC  
 406▶ Met Ser Gl uTyrProThr TyrHi sThr Hi sGl yA rgTyrVal l Pro  
 1261 CCTAGCAGTACCGATCGTAGCCCTATGAGAAGGTTTCTGCAGGT  
 421▶ ProSer Ser ThrAspArgSer ProTyrGl uLysVal Ser Al aGl y  
 1306 AATGGTGGCAGCAGCCTCTCTTACACAAACCCAGCAGTGGCAGCC  
 436▶ AsnGl yGl ySer SerLeuSer TyrThrAsnProAl aVal Al aAl a  
 1351 ACTTCTGCCAACTTGTGATAA  
 451▶ Thr Ser Al aAsnLeu.....

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Figure 6

1 ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC  
1▶MetGlnIlePheValLysThrLeuThrGlyLysThrIleThrLeu  
46 GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC  
16▶GluValGluProSerAspThrIleGluAsnValLysAlaLysIle  
91 CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT  
31▶GlnAspLysGluGlyIleProProAspGlnGlnArgLeuIlePhe  
136 GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC  
46▶AlaGlyLysGlnLeuGluAspGlyArgThrLeuSerAspTyrAsn  
181 ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT  
61▶IleGlnLysGluSerThrLeuHisLeuValLeuArgLeuArgGly  
226 GGGAGGCACGGTAGTGGTGCAATGGCTGTTGCCCGTCTCGCTGGTG  
76▶GlyArgHisGlySerGlyAlaTrpLeuLeuProValSerLeuVal  
271 AAAAGAAAAACCAACCTGGCGCCCAATACGCAAACCGCTCTCCC  
91▶LysArgLysThrThrLeuAlaProAsnThrGlnThrAlaSerPro  
316 CGCGCGTTGGCCGATTCATTAATGCAGCTGGCAGCAGAGGTTTCC  
106▶ArgAlaLeuAlaAspSerLeuMetGlnLeuAlaArgGlnValSer  
361 CGAGGATCC  
121▶ArgGlySer

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Figure 7

1 ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC  
1▶Met Gl n l l ePheVal LysThr LeuThr GlyLysThr l l eThr Leu  
46 GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGCCAAAGATC  
16▶Gl uVal Gl uProSer AspThr l l eGl uAsnVal LysAl aLys l l e  
91 CAAGACAAGGAAGGCATCCCCTCCTGACCAGCAGAGGCTCATCTTT  
31▶Gl nAspLysGl uGl y l l eProProAspGl nGl nArgLeu l l ePhe  
136 GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC  
46▶Al aGl yLysGl nLeuGl uAspGl yA rgThr LeuSer AspTyrAsn  
181 ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT  
61▶l l eGl nLysGl uSer Thr LeuHi sLeuVal LeuArgLeuArgGl y  
226 GGGAGGCACGGTAGTGGTGTCATGGCTGTTGCCCGTCTCGCTGGTG  
76▶Gl yA rgHi sGl ySer Gl yAl aT rpLeuLeuProVal Ser LeuVal  
271 AAAAGAAAAACCACCCTGGCGCCAATACGCAAACCGCCTCTCCC  
91▶LysArgLysThr Thr LeuAl aProAsnThr Gl nThr Al aSer Pro  
316 CGCGCGTTGGCCGATTTCATTAATGCAGCTGGCAGCAGAGTTTCC  
106▶A rgAl aLeuAl aAspSer LeuMet Gl nLeuAl aArgGl nVal Ser  
361 CGAGGATCCACAGGTTCTGGTCATGCAAGCTCTACCCAGGTGGA  
121▶A rgGl ySer Thr Gl ySer Gl yHi sAl aSer Ser Thr ProGl yGl y  
406 GAAAAGGAGACTTCGGCTACCCAGAGAAGTTTCAGTGCCAGCTCT  
136▶Gl uLysGl uThr Ser Al aThr Gl nArgSer Ser Val ProSer Ser  
451 ACTGAGAAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGC  
151▶Thr Gl uLysAsnAl aVal SerMet Thr Ser Ser Val LeuSer Ser  
496 CACAGCCCCGGTTCAGGCTCCTCCACCCTCAGGGACAGGATGTCT  
166▶Hi sSer ProGl ySer Gl ySer Ser Thr Thr Gl nGl yGl nAspVal  
541 ACTCTGGCCCCGGCCACGGAACCACTTCAGGTTGATAA  
181▶Thr LeuAl aProAl aThr Gl uProAl aSer Gl y.....

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Figure 8

1 ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC  
 1>Met Gl n l l ePheVal LysThr LeuThr Gl yLysThr l l eThr Leu  
 46 GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAGATC  
 16>Gl uVal l Gl uProSer AspThr l l eGl uAsnVal l LysAl aLys l l e  
 91 CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT  
 31>Gl nAspLysGl uGl y l l eProProAspGl nGl nArgLeu l l ePhe  
 136 GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTCTGACTACAC  
 46>Al aGl yLysGl nLeuGl uAspGl yA rgThr LeuSerAspTyrAsn  
 181 ATCCAGAAAGAGTCCACCCTGCACCTGGTGTCTCCGTCTCAGAGGT  
 61> l l eGl nLysGl uSer Thr LeuHi sLeuVal l LeuArgLeuArgGl y  
 226 GGGAGGCACGGTAGTGGTGCATGGCTGTGCCCCGTCTCGCTGGTG  
 76>Gl yA rgHi sGl ySer Gl yAl aT rpLeuLeuProVal Ser LeuVal  
 271 AAAAGAAAAACCACCTGGCGCCCAATACGCAAAACCGCCTCTCCC  
 91>Lys ArgLysThr Thr LeuAl aProAsnThr Gl nThr Al aSer Pro  
 316 CGCGCGTTGGCCGATTCTTAATGCAGCTGGCAGCAGAGTTTCC  
 106>A rgAl aLeuAl aAspSer LeuMet Gl nLeuAl aArgGl nVal Ser  
 361 CGAGGATCCGTGCCAGCTCTACTGAGAAGAATGCTGTGAGTATG  
 121>A rgGl ySer Val ProSer Ser Thr Gl uLysAsnAl aVal SerMet  
 406 ACCAGCAGCGTACTCTCCAGCCACAGCCCCGGTTCAGGCTCTCTCC  
 136>Thr Ser Ser Val l LeuSer Ser Hi sSer ProGl ySer Gl ySer Ser  
 451 ACCACTCAGGGACAGGATGTCACTCTGGCCCCGGCCACGGAACCA  
 151>Thr Thr Gl nGl yGl nAspVal Thr LeuAl aProAl aThr Gl uPro  
 496 GCTTCAGGTTCAAGTGCACCTGGGGACAGGATGTCACTCCGTC  
 166>Al aSer Gl ySer Al aAl aThr T rpGl yGl nAspVal Thr Ser Val  
 541 CCAGTCACCAGGCCAGCCCTGGGCTCCACCACCCCGCCAGCCAC  
 181>ProVal Thr ArgProAl aLeuGl ySer Thr Thr ProProAl aHi s  
 586 GATGTCACTCAGCCCCGACACAACAAGCCAGCCCCGGGAAGTACT  
 196>AspVal Thr Ser Al aProAspAsnLysProAl aProGl ySer Thr  
 631 GCTCCACAGCACAGCGTGTACCTCGGCTCCGGATACACGGCG  
 211>Al aProProAl aHi sGl yVal Thr Ser Al aProAspThr ArgPro  
 676 GCCCAGGTAGTACCGCCCTCTGCCCATTGGTGTACATCTGCC  
 226>Al aProGl ySer Thr Al aProProAl aHi sGl yVal Thr Ser Al a  
 721 CGGCAACACAGGCCTGCATTGGGTAGTACAGCACGCCAGTACAC  
 241>ProAspAsnArgProAl aLeuGl ySer Thr Al aProProVal l Hi s  
 766 AACGTTACTAGTGCTCAGGCTCTGCTAGCGGCTCAGCTTCTACT  
 256>AsnVal Thr Ser Al aSer Gl ySer Al aSer Gl ySer Al aSer Thr  
 811 CTGGTGACACAACGGCACCTCTGCGCGCGGACCAACCCAGCG  
 271>LeuVal l Hi sAsnGl yThr Ser Al aArgAl aThr Thr Thr ProAl a  
 856 ACCAAGAGCACTCCATTCTCAATTCCCAGCTGATAA  
 286>Ser LysSer Thr ProPheSer l l eProSer •••••

Figure 9

1 ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC  
 1▶ Met Gl n I l e Phe Val Lys Thr Leu Thr Gly Lys Thr I l e Thr Leu  
 46 GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC  
 16▶ Gl u Val Gl u Pro Ser Asp Thr I l e Gl u Asn Val Lys Al a Lys I l e  
 91 CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT  
 31▶ Gl n Asp Lys Gl u Gl y I l e Pro P ro Asp Gl n Gl n Arg Leu I l e Phe  
 136 GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTCTGACTACAAC  
 46▶ Al a Gl y Lys Gl n Leu Gl u Asp Gl y A rg Thr Leu Ser Asp Tyr Asn  
 181 ATCCAGAAAGAGTCCACCCTGCACCTGGTGCCTCCGTCTCAGAGGT  
 61▶ I l e Gl n Lys Gl u Ser Thr Leu Hi s Leu Val Leu Arg Leu Arg Gl y  
 226 GGGAGGCACGGTAGTGGTGCATGGCTGTGGCCGCTCTCGCTGGTG  
 76▶ Gly A rg Hi s Gl y Ser Gl y Al a T rp Leu Leu Pro Val Ser Leu Val  
 271 AAAAGAAAAACCACCCTGGCGCCCAATACGCAAAACCGCCTCTCCC  
 91▶ Lys Arg Lys Thr Thr Leu Al a P ro Asn Thr Gl n Thr Al a Ser Pro  
 316 CGCGCGTTGGCCGATTCAATATGCAGCTGGCACACAGGTTTCC  
 106▶ A rg Al a Leu Al a Asp Ser Leu Met Gl n Leu Al a Arg Gl n Val Ser  
 361 CAGGATCCGGCTCAGCTTCTACTCTGGTGCAACCGCACTCT  
 121▶ A rg Gl y Ser Gl y Ser Al a Ser Thr Leu Val Hi s Asn Gl y Thr Ser  
 406 GCCAGGCTACCAACCCAGCCAGCAAGAGCACTCCATTCTCA  
 136▶ Al a Arg Al a Thr Thr Thr Pro Al a Ser Lys Ser Thr Pro Phe Ser  
 451 ATTCCAGCCCACTCTGATACTCTACCACCCTTGCCAGGCAT  
 151▶ I l e Pro Ser Hi s Hi s Ser Asp Thr Pro Thr Thr Leu Al a Ser Hi s  
 496 AGCACAAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCT  
 166▶ Ser Thr Lys Thr Asp Al a Ser Ser Thr Hi s Hi s Ser Thr Val Pro  
 541 CCTCTCACTCTCCAATCACAGCACTTCTCCCCAGTTGTCTACT  
 181▶ Pro Leu Thr Ser Ser Asn Hi s Ser Thr Ser Pro Gl n Leu Ser Thr  
 586 GGGTCTCTTTCTTTTCTGTCTTTTCACATTTCAAACCTCCAG  
 196▶ Gl y Val Ser Phe Phe Phe Leu Ser Phe Hi s I l e Ser Asn Leu Gl n  
 631 TTTAATTCCTCTCTGGAAGATCCCAAGCACCAGTACTACCAAGAG  
 211▶ Phe Asn Ser Ser Leu Gl u Asp Pro Ser Thr Asp Tyr Tyr Gl n Gl u  
 676 CTGCAGAGAGACATTTCTGAATGTTTTGCGAGATTATAACAA  
 226▶ Leu Gl n Arg Asp I l e Ser Gl u Met Phe Leu Gl n I l e Tyr Lys Gl n  
 721 GGGGTTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCT  
 241▶ Gl y Gl y Phe Leu Gl y Leu Ser Asn I l e Lys Phe Arg Pro Gl y Ser  
 766 GTGGTGGTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAAT  
 256▶ Val Val Val Gl n Leu Thr Leu Al a Phe Arg Gl u Gl y Thr I l e Asn  
 811 GTCCACGACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCA  
 271▶ Val Hi s Asp Val Gl u Thr Gl n Phe Asn Gl n Tyr Lys Thr Gl u Al a  
 856 GCCTCTCGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGAT  
 286▶ Al a Ser A rg Tyr Asn Leu Thr I l e Ser Asp Val Ser Val Ser Asp  
 901 GTGCCATTTCCTTTCTCTGCCAGTCTGGGGCTGGGGTGCCAGGC  
 301▶ Val Pro Phe Pro Phe Ser Al a Gl n Ser Gl y Al a Gl y Val Pro Gl y  
 946 TGGGGCATCGCGCTGCTGGTGTCTGGTCTGTGTCTGGTGTGGCTG  
 316▶ T rp Gl y I l e Al a Leu Leu Val Leu Val Cys Val Leu Val Al a Leu  
 991 GCCATTGTCTATCTCATTCCTTGTGATAA  
 331▶ Al a I l e Val Tyr Leu I l e Al a Leu.....

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Figure 10

1 ATGCAGATCTTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC  
 1▶ Met Gl n l l e Phe Val Lys Thr Leu Thr Gl y Lys Thr l l e Thr Leu  
 46 GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAGATC  
 16▶ Gl u Val Gl u Pro Ser Asp Thr l l e Gl u Asn Val Lys Al a Lys l l e  
 91 CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT  
 31▶ Gl n Asp Lys Gl u Gl y l l e P ro P ro Asp Gl n Gl n Arg Leu l l e Phe  
 136 GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC  
 46▶ Al a Gl y Lys Gl n Leu Gl u Asp Gl y A rg Thr Leu Ser Asp Tyr Asn  
 181 ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT  
 61▶ l l e Gl n Lys Gl u Ser Thr Leu His Leu Val Leu Arg Leu Arg Gl y  
 226 GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG  
 76▶ Gl y A rg His Gl y Ser Gl y Al a Trp Leu Leu Pro Val Ser Leu Val  
 271 AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC  
 91▶ Lys Arg Lys Thr Thr Leu Al a Pro Asn Thr Gl n Thr Al a Ser Pro  
 316 CGCGCGTTGGCCGATTCAATTAATGCAGCTGGCAGCAGAGTTTCC  
 106▶ A rg Al a Leu Al a Asp Ser Leu Met Gl n Leu Al a Arg Gl n Val Ser  
 361 CGAGGATCCCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATT  
 121▶ A rg Gl y Ser Leu Val Leu Val Cys Val Leu Val Al a Leu Al a l l e  
 406 GTCTATCTCATTGCCCTGGCTGTCTGTCTCAGTGCCGCCGAAAGAAC  
 136▶ Val Tyr Leu l l e Al a Leu Al a Val Cys Gl n Cys Arg Arg Lys Asn  
 451 TACGGGCAGCTGGACATCTTTCCAGCCCGGGATACCTACCATCCT  
 151▶ Tyr Gl y Gl n Leu Asp l l e Phe Pro Al a Arg Asp Thr Tyr His S Pro  
 496 ATGACCGAGTACCCACCTACCACCCCATGGCGCTATGGTGCC  
 166▶ Met Ser Gl u Tyr Pro Thr Tyr His S Thr His Gl y A rg Tyr Val Pro  
 541 CCTAGCAGTACCGATCGTAGCCCTATGAGAAGGTTTCTGCAGGT  
 181▶ Pro Ser Ser Thr Asp Arg Ser Pro Tyr Gl u Lys Val Ser Al a Gl y  
 586 AATGGTGGCAGCAGCCTCTCTTACACAAACCCAGCAGTGGCAGCC  
 196▶ Asn Gl y Gl y Ser Ser Leu Ser Tyr Thr Asn Pro Al a Val Al a Al a  
 631 ACTTCTGCCAACTTGTGATAA  
 211▶ Thr Ser Al a Asn Leu • • • • •

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Figure 11

1 ATGCAGATCTTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC  
 1▶ Met Gl n l l e Phe Val Lys Thr Leu Thr Gl y Lys Thr l l e Thr Leu  
 46 GAAGTGGAGCCGAGTGACACCATTTGAGAATGTCAAGGCAAAGATC  
 16▶ Gl u Val Gl u Pro Ser Asp Thr l l e Gl u Asn Val Lys Al a Lys l l e  
 91 CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT  
 31▶ Gl n Asp Lys Gl u Gl y l l e Pro Pro Asp Gl n Gl n Arg Leu l l e Phe  
 136 GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTCTGACTACAAC  
 46▶ Al a Gl y Lys Gl n Leu Gl u Asp Gl y A rg Thr Leu Ser Asp Tyr Asn  
 181 ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT  
 61▶ l l e Gl n Lys Gl u Ser Thr Leu Hi s Leu Val Leu Arg Leu Arg Gl y  
 226 GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG  
 76▶ Gl y A rg Hi s Gl y Ser Gl y Al a T rp Leu Leu Pro Val Ser Leu Val  
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 361 CGAGGATCCACAGGTTCTGGTCATGCAAGCTCTACCCAGGTGGA  
 121▶ A rg Gl y Ser Thr Gl y Ser Gl y Hi s Al a Ser Ser Thr Pro Gl y Gl y  
 406 GAAAAGGAGACTTCGGCTACCCAGAGAAGTTCACTGCCAGCTCT  
 136▶ Gl u Lys Gl u Thr Ser Al a Thr Gl n Arg Ser Ser Val Pro Ser Ser  
 451 ACTGAGAAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGC  
 151▶ Thr Gl u Lys Asn Al a Val Ser Met Thr Ser Ser Val Leu Ser Ser  
 496 CACAGCCCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTC  
 166▶ Hi s Ser Pro Gl y Ser Gl y Ser Ser Thr Thr Gl n Gl y Gl n Asp Val  
 541 ACTCTGGCCCCGGCCACGGAACCAAGCTTCAGGTTCACTGTCACC  
 181▶ Thr Leu Al a Pro Al a Thr Gl u Pro Al a Ser Gl y Ser Al a Al a Thr  
 586 TGGGGACAGGATGTCACCTCGGTCCCACTCACCAGGCAGCCCTG  
 196▶ T rp Gl y Gl n Asp Val Thr Ser Val Pro Val Thr Arg Pro Al a Leu  
 631 GGCTCCACCACCCCGCCAGCCACGATGTACCTCAGCCCCGAC  
 211▶ Gl y Ser Thr Thr Pro Pro Al a Hi s Asp Val Thr Ser Al a Pro Asp  
 676 AACAAGCCAGCCCCGGGAAGTACCGCTCCACCAGCACAGCGTGT  
 226▶ Asn Lys Pro Al a Pro Gl y Ser Thr Al a Pro Pro Al a Hi s Gl y Val  
 721 ACCTCGGCTCCGATACCAAGGCCGCCCCAGGTAGTACCGCCCT  
 241▶ Thr Ser Al a Pro Asp Thr A rg Pro Al a Pro Gl y Ser Thr Al a Pro  
 766 CTGCCCCATGGTGTACATCTGCCCCGACAAACGGCCCTGCATTG  
 256▶ Pro Al a Hi s Gl y Val Thr Ser Al a Pro Asp Asn Arg Pro Al a Leu  
 811 GGTAGTACAGCACCGCCAGTACACAACGTTACTAGTGCCTCAGGC  
 271▶ Gl y Ser Thr Al a Pro Pro Val Hi s Asn Val Thr Ser Al a Ser Gl y  
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 286▶ Ser Al a Ser Gl y Ser Al a Ser Thr Leu Val Hi s Asn Gl y Thr Ser

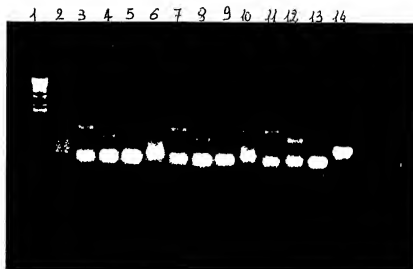
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Figure 11 (continued)

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 946 ATTCCCAGCCACCACTCTGATACTCCTACCACCCTTGCCAGCCAT  
 316▶IleProSerHisHisSerAspThrProThrThrLeuAlaSerHis  
 991 AGCACCAAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCT  
 331▶SerThrLysThrAspAlaSerSerThrHisHisSerThrValPro  
 1036 CCTCTCACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTCTACT  
 346▶ProLeuThrSerSerAsnHisSerThrSerProGlnLeuSerThr  
 1081 GGGGTCTCTTTCTTTTCTGTCTTTTCACATTTCAAACCTCCAG  
 361▶GlyValSerPhePhePheLeuSerPheHisIleSerAsnLeuGln  
 1126 TTTAATTCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAG  
 376▶PheAsnSerSerLeuGluAspProSerThrAspTyrTyrGlnGlu  
 1171 CTGCAGAGAGACATTTCTGAAATGTTTTGCAGATTTATAAACAA  
 391▶LeuGlnArgAspIleSerGluMetPheLeuGlnIleTyrLysGln  
 1216 GGGGGTTTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCT  
 406▶GlyGlyPheLeuGlyLeuSerAsnIleLysPheArgProGlySer  
 1261 GTGGTGGTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAAT  
 421▶ValValValGlnLeuThrLeuAlaPheArgGluGlyThrIleAsn  
 1306 GTCCACGACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCA  
 436▶ValHisAspValGluThrGlnPheAsnGlnTyrLysThrGluAla  
 1351 GCCTCTCGATATAACCTGACGATCTCAGACGTACGCGTGAGTGAT  
 451▶AlaSerArgTyrAsnLeuThrIleSerAspValSerValSerAsp  
 1396 GTGCCATTCTCTTTCTCTGCCAGTCTGGGGCTGGGGTCCGAGC  
 466▶ValProPheProPheSerAlaGlnSerGlyAlaGlyValProGly  
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 481▶TrpGlyIleAlaLeuLeuValLeuValCysValLeuValAlaLeu  
 1486 GCCATTGTCTATCTCATTGCCTTGGCTGTCTGTCACTGCGCCGA  
 496▶AlaIleValTyrLeuIleAlaLeuAlaValCysGlnCysArgArg  
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 511▶LysAsnTyrGlyGlnLeuAspIlePheProAlaArgAspThrTyr  
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 526▶HisProMetSerGluTyrProThrTyrHisThrHisGlyArgTyr  
 1621 GTGCCCCCTAGCAGTACCGATCGTAGCCCCCTATGAGAAGGTTTCT  
 541▶ValProProSerSerThrAspArgSerProTyrGluLysValSer  
 1666 GCAGGTAATGGTGGCAGCAGCCTCTCTACACAAACCCAGCAGTG  
 556▶AlaGlyAsnGlyGlySerSerLeuSerTyrThrAsnProAlaVal  
 1711 GCAGCCACTTCTGCCAAGTTGTGATAA  
 571▶AlaAlaThrSerAlaAsnLeu.....

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Figure 13

1 CCAGGAAGCTCCTCTGTGCTCTATAAACCCCTAACCTCCTCTACTTGTAGA  
51 GGACATTCCAATCATAGGCTGCCCATCCACCCTCTGTGTCCTCCTGTAA  
101 TTAGGTCACTTAACAAAAAGGAAATTGGTAGGGTTTTTCACAGACGCG  
151 TTTCTAAGGGTAATTTTAAAAATATCTGGGAAGTCCCTTCCACTGCTGTGT  
201 TCCAGAAGTGTGGTAAACAGCCCAAAATGTCAACAGCAGAAACATACA  
251 AGCTGTCAGCTTTGCACAAGGGCCCAACCCCTGCTCATCAAGAAGCACT  
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501 TTGAGCAGGATATTTGGTCTGTAGTTTGCTAACACACCCTGCAGCTCCA  
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651 CATAGCAGTTACCCCAATAACCTCAGTTTTTAACAGTAACAGCTTCCACA  
701 TCAAAATATTTCCACAGGTTAAGTCTCATTTAAATTAGGCAAAAGGAATT  
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851 GCGCGGAACCCCTATTTGTTTATTTTCTAAATACATTCAAATATGTATC  
901 CGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGG  
951 AAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTGC  
1001 GGCATTTTGCCTTCTGTTTTGTCTACCCAGAAACGCTGGTGAAAGTAA

Figure 13

2151 TAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGC  
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2251 TTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTG  
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3051 CATTAGTTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTA  
3101 AATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCATAT  
3151 AATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC  
3201 AATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTG  
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(Continued)

Figure 13 (Continued)

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(Continued)

Figure 13 (Continued)

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1201 TTGACGCGGGCAAGAGCAACTCGGTGCGCATACACTATTCTCAGAAT  
1251 GACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCAT  
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1451 GGAGCTGAATGAAGCCATACCAAACGAGCGGTGACACCAAGATGCGCTG  
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1901 TTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCTGTCCACT  
1951 GAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT  
2001 TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACACG  
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(Continued)

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Figure 13 (Continued)

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&lt;211&gt; 369

&lt;212&gt; DNA

&lt;213&gt; human

&lt;400&gt; 6

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&lt;210&gt; 7

&lt;211&gt; 579

&lt;212&gt; DNA

&lt;213&gt; human

&lt;400&gt; 7

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<211> 4905

<212> DNA

<213> human

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4905

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&lt;211&gt; 31

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
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31

&lt;210&gt; 14

&lt;211&gt; 41

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

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41

&lt;210&gt; 15

&lt;211&gt; 36

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
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36

&lt;210&gt; 16

&lt;211&gt; 49

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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<210> 17

<211> 40

<212> DNA

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<210> 21

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<210> 23

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<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 23

gatctctaga atgggtctcag cttctactct ggtgcacaac ggc

43

<210> 24

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 24

gatctctaga atcggtgtgc tggctctgtg tctggttgcg c

41

<210> 25

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 25

ggcgggtggag cccggggctg gcttgc

26

<210> 26

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 26

aacctgaagc tggttccgtg gc

22

<210> 27

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic

## oligonucleotide

&lt;400&gt; 27

gtgccagct ctactgagaa gaatgc

26

&lt;210&gt; 28

&lt;211&gt; 29

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

&lt;400&gt; 28

gctgggaatt gagaatggag tgctcttgc

29

&lt;210&gt; 29

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

&lt;400&gt; 29

ggctcagctt ctactctggt gcacaacggc

30

&lt;210&gt; 30

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

&lt;400&gt; 30

caaggcaatg agatagacaa tggcc

25

&lt;210&gt; 31

&lt;211&gt; 27

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 31

ctggtgctgg tctgtgttct ggtgcg

27

<210> 32

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 32

gatctctaga atgcagatct tcgtgaagac cctgactggt

40

<210> 33

<211> 68

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 33

tcaccagcga gacgggcaac agccatgcac cactaccgtg cctccacct ctgagacgga 60  
gcaccagg 68

<210> 34

<211> 66

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
oligonucleotide

<400> 34

ccctcgctctc agaggtggga ggcacggtag tggatcatgg ctgttgcccg tctcgctggt 60

gaaaag

66

&lt;210&gt; 35

&lt;211&gt; 35

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<221> Description of Artificial Sequence: synthetic  
oligonucleotide

&lt;400&gt; 35

gatcggatcc tcgggaaacc tgtcgtgccca gctgc

35